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Molecular genetic analysis of the model organism *Caenorhabditis elegans* was used to identify and study mechanisms of action of negative regulators of tyrosine kinase/RAS mediated signal transduction that are candidate tumor suppressors. A homolog of the proto oncogene *cbl,* SLI-1, inhibits Ras activation by the epidermal growth factor receptor homolog LET-23. Three functional domains of SLI-1 have been identified. The ARK-1 protein kinase was discovered and shown to inhibit signaling by LET-23. New screens for additional negative regulators have identified at several genes that will be molecularly cloned.

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PI - Signature Date

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Introduction

The previous year's report was reviewed as if it were a final report, even though there was a no-cost extension. This report serves as a brief addendum to the last report.

Body

The specific goals of the project are as follows.

1. **Analyze SLI-1 function** in *C. elegans* through molecular genetics.
2. **Molecularly clone *sli-2*.**
3. **Molecularly clone *rok-1*.**
4. **Identify and clone additional genes acting in concert with *sli-1*, *sli-2*, and *rok-1***
5. **Examine the functional interactions of *sli-1*, *sli-2*, *rok-1* in regulating other conserved signaling pathways.**
6. **Clone human *sli-2*, *rok-1*, and newly identified genes from human breast tissue libraries** to generate reagents with which to test the hypothesis that these are novel tumor suppressor loci.
7. **Test the functional homology of *c-cbl* and *sli-1*** by introducing the human cDNA into transgenic nematodes defective in *sli-1*.

1. *sli-1*.

Completed.

2. *sli-2*.

Our analysis of *sli-2* is completed except for the molecular cloning. The main new genetic data is a test of the signal dependence of vulval differentiation in *sli-2* mutants. The excessive vulval differentiation displayed by *sli-2* in combination with other negative regulators is dependent on the inductive signal originating from the hermaphrodite gonad (Table1).

Table 1. *sli-2* hyperinduction is gonad dependent. Gonadal precursors were ablated in the indicated number of animals of each genotype, and the extent of vulval differentiation scored in the late L3 or L4 larval stages by examination with Nomarski optics.

	Gonad (+)	Gonad (-)
sli-2(sy262)	3.0 (n=30)	0.0 (n=6)
let-23(sy1); sli-2(sy262)	3.9 (n=31)	0.0 (n=5)
sli-2(sy262); gap-1(n1691)	3.3 (n=20)	0.0 (n=8)
unc-101(sy108); sli-2(sy262)	3.2 (n=20)	0.0 (n=6)

3. Genetics and molecular cloning of *rok-1*

Completed.
A paper on *rok-1*, renamed *ark-1* (Ack-related kinase) in response to reviewers, is being revised for the journal Cell.

4. Identification and cloning of additional negative regulators

As described in previous reports, we have identified new negative regulators of LET-23 - RAS signaling. We will continue their analysis.

The mutation 46-1 isolated as an enhancer of the multivulva phenotype of *let-23(sa62)*/+ has been mapped to Linkage Group IV between *unc-24* and *dpy-20*. The map position will be refined and this locus cloned.

5. Gene interactions

Completed.

6. Human homologs

Completed. We failed to identify a human homolog of *rok-1* (*ark*-1).

7. Human cbl in *C. elegans*.

Completed.

Conclusions

Analysis of SLI-1, C. elegans homolog of Cbl, revealed functionally important domains.

Discovery of ARK-1 an Ack-related protein kinase involved in negative regulation of LET-23 signaling. Genetic analysis of ARK-1 suggests that it is recruited to the LET-23 signaling complex by the adaptor SEM-5.

New regulatory genes were discovered as suppressors or enhancers of existing mutations.

Progress by task as per original Statement of Work:

A brief description of progress on each task is listed.

Task 1A. Determine whether SLI-1 truncation decreases or increases activity of the protein as assayed in transgenic animals. •[Completed]

Task 1B. Determine role of alternative spliced form of SLI-1. •[Completed].

Task 1C. *sli-1* point mutation sequencing •[Completed].

Task 1D. *sli-1* antisera. [not completed].

Task 2A Genetic characterization of *sli-2*. [completed]

Task 2B. Molecular cloning of SLI-2 from *C. elegans*. [not completed]

Task 3. Genetics and molecular cloning of ROK-1 from *C. elegans*. [Completed]

Task 4. Identification by genetic screens of new loci.

a. Screen for new mutations, carry out screens in parallel. [completed]

b. Genetic mapping and complementation of new mutations, parallel experiments •[completed]

c. Molecular cloning [not completed]

Task 5. Examination interactions of genes in vivo •[completed]

Task 6. Human homologs. •[unsuccessful]

Task 7. Introduction of c-cbl cDNA into transgenic nematodes. a. **Construct *sli-1/c-cbl* hybrid genes b. Examine phenotypes of transgenic animals.** • [completed].